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# UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No. ST94037A-US

First Inventor or Application Identifier Carillo et al.

Title Method of Cancer Treatment by P53 Protein Control

Express Mail Label No. EE458494950US

## APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

- ☒ \* Fee Transmittal Form (e.g., PTO/SB/17)  
(Submit an original and a duplicate for fee processing)
- ☒ Specification [Total Pages 34]  
(preferred arrangement set forth below)
  - Descriptive title of the Invention
  - Cross References to Related Applications
  - Statement Regarding Fed sponsored R & D
  - Reference to Microfiche Appendix
  - Background of the Invention
  - Brief Summary of the Invention
  - Brief Description of the Drawings (if filed)
  - Detailed Description
  - Claim(s)
  - Abstract of the Disclosure
- ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets 1]
- Oath or Declaration [Total Pages 3]
  - ☐ Newly executed (original or copy)
  - ☒ Copy from a prior application (37 C.F.R. § 1.63(d))  
(for continuation/divisional with Box 16 completed)
    - ☐ DELETION OF INVENTOR(S)  
Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

**\* NOTE FOR ITEMS 1 & 13: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28).**

ADDRESS TO: Assistant Commissioner for Patents  
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Washington, DC 20231

- ☐ Microfiche Computer Program (Appendix)
- Nucleotide and/or Amino Acid Sequence Submission  
(if applicable, all necessary)
  - ☒ Computer Readable Copy
  - ☒ Paper Copy (identical to computer copy)
  - ☒ Statement verifying identity of above copies

## ACCOMPANYING APPLICATION PARTS

- ☐ Assignment Papers (cover sheet & document(s))
- ☐ 37 C.F.R. § 3.73(b) Statement of Power of Attorney  
(when there is an assignee)
- ☐ English Translation Document (if applicable)
- ☐ Information Disclosure Statement (IDS)/PTO-1449  
Copies of IDS Citations
- ☒ Preliminary Amendment
- ☒ Return Receipt Postcard (MPEP 503)  
(Should be specifically itemized)
- ☐ \* Small Entity Statement(s) filed in prior application,  
(PTO/SB/09-12) Status still proper and desired
- ☐ Certified Copy of Priority Document(s)  
(if foreign priority is claimed)
- ☒ Other: Associate Power of Attorney

16. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment:

☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No: 08,737,953  
Prior application information: Examiner B. Campell Group / Art Unit: 1632

**For CONTINUATION or DIVISIONAL APPS only:** The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

## 17. CORRESPONDENCE ADDRESS

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# **CONTINUATION APPLICATION FOR PATENT COVER SHEET**

## CERTIFICATE OF MAILING (37 CFR 1.10)

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September 24, 1999  
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Paula L. Dickey  
(Type or print name of person mailing paper)

Paula L. Dickey  
(Signature of person mailing paper)

Transmitted herewith are the following:

1. Utility Patent Application Transmittal  
Continuation of prior application No. 08/737,953;
2. Fee Transmittal Sheet;
3. Associate Power of Attorney;
4. Copy of Declaration and Power of Attorney;
5. Preliminary Amendment with copy of Sequence Listing;
6. Specification 30 pages;  
Claims 3 pages;  
Abstract 1 pages;  
Drawings 1 pages;
7. Paper Copy of Sequence Listing with Computer Readable disk.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: CARILLO *et al.* Group Art Unit:  
Serial No.: To Be Assigned Examiner:  
U.S. National Stage of PCT/FR95/00670  
Filed: Concurrently Herewith

For: Method of Cancer Treatment By P53 Protein Control

To: The Honorable Commissioner of Patents and Trademarks  
Washington, D.C. 20231

CERTIFICATE OF MAILING (37 CFR § 1.10)

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(Signature of person mailing paper)

**PRELIMINARY AMENDMENT**

Please enter the following amendment to the English Translation of the International Application before examining this application.

**In the Specification:**

At page 1, line 3, please insert "This application is a Continuation Application of co-pending US National Stage Application No. 08/737,953, filed November 27, 1996."

At page 5, line 24, delete --No. 1-- and insert therefore "Nos. 1 and 2".

At page 6, line 6, delete --No. 1-- and insert therefore "Nos. 1 and 2".

At page 7, line 11, delete --No. 1-- and insert therefore "No. 2".

At page 7, line 15, delete --No. 2-- and insert therefore "Nos. 3 and 4".

At page 7, lines 17-18, delete --1 or 2-- and insert therefore "2 or 4".

Please replace pages 26-30 with the attached SUBSTITUTE SEQUENCE LISTING (pages 26-32), and renumber the claims and abstract pages accordingly.

**In the Claims:**

Please cancel claims 1-17 without prejudice.

Please add the following new claims (claims 18-29):

18. A method for regulating cellular levels of p53 protein comprising administering to cells a vector comprising a nucleic acid encoding a protein or polypeptide, wherein the protein or polypeptide is an inhibitor of the activity of calpain, wherein the encoded protein or polypeptide inhibits the activity of calpain upon its expression in the cells, thereby regulating cellular levels of p53 protein.

19. The method according to claim 18, wherein the vector is a viral vector selected from the group consisting of adenoviruses, retroviruses and adeno-associated viruses.

20. The method according to claim 18, wherein the vector is a lipid liposomal vector.

21. The method according to claim 18, wherein the nucleic acid encodes all or part of calpastatin.

22. The method according to claim 21, wherein the nucleic acid comprises all or part of sequence SEQ ID No. 1.

23. The method according to claim 21, wherein the nucleic acid has a sequence selected from the group consisting of SEQ ID No. 1 and SEQ ID No. 3.

24. The method according to claim 18, wherein the nucleic acid encodes a protease inhibitor.

25. The method according to claim 24, wherein the protease inhibitor is leupeptin.

26. A viral vector comprising a nucleic acid encoding a protein or polypeptide, wherein the protein or polypeptide is an inhibitor of the activity of calpain.

27. The vector according to claim 26, selected from the group consisting of adenoviruses, retroviruses and adeno-associated viruses.

28. The vector according to claim 26, comprising a sequence encoding all or part of calpastatin.

29. A composition comprising a nucleic acid encoding all or part of calpastatin that has the capacity to inhibit, at least in part, calpain, formulated for intra-tumor administration.

“calpain” inhibitor

**REMARKS**

Applicants submit a SEQUENCE LISTING in compliance with the requirements of 37 C.F.R. §§ 1.821-1.825. The Specification has been amended to recite sequence identifiers corresponding to the SEQUENCE LISTING. No new matter has been added.

Claims 1-17 have been cancelled and rewritten as new claims 18-29 to conform with US patent practice. Support for the new claims is found in the claims as originally filed and in the Specification. No new matter has been added.

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Respectfully submitted,



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Provisional Reg. No. P-45,052

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## METHOD OF CANCER TREATMENT BY P53 PROTEIN CONTROL

The present invention relates to a new method for the treatment of cancer. More particularly, it relates to a method of treating cancer by regulating the cellular levels of the p53 protein. It also relates to vectors for gene therapy which make it possible to regulate the p53 protein, as well as the pharmaceutical compositions containing them.

For the past fifteen years, the molecular characterization of oncogenes and of tumour suppressor genes has made it possible to view the process of carcinogenesis in a new light. Thus, the increasingly detailed knowledge of the regulation of these genes and of the function of the corresponding proteins makes it possible to conceive new therapeutic approaches.

More particularly, the elucidation of the breakdown of the oncogenic and anti-oncogenic proteins represents a major challenge in terms of the fight against cancer since it presages, in the case of oncogenic proteins, the possibility of accelerating their degradation and therefore of annihilating their action, in the case of tumour suppressors, inhibiting their degradation and therefore increasing their antiproliferative or anti-tumour effect, in the case of mutated proteins, potentiating their antigenic presentation by molecules of the Major

Histocompatibility Complex and thereby stimulating a tumour-specific immune response, and, in the case where the high expression of the oncogene or of the anti-oncogene is capable of inducing programmed cell death, the possibility of stabilizing these proteins so as to trigger the apoptotic process.

Originally, the p53 protein was classified as a nuclear oncogene since it could, in transfection experiments, extend the life of rodent cells in culture as well as cooperate with activated oncogenes such as ras to transform cells in primary culture. Indeed, the genes used in these first experiments were mutated and led to the expression of variant p53 proteins characterized by a gain in function. Without excluding functions which might still be discovered, it is now known that the p53 protein, at least in its wild-type form, is a transcription factor which negatively regulates growth and cell division and which, in certain situations, is capable of inducing apoptosis (Yonish-Rouach et al., Nature, 352, 345-347, 1991). Given that these properties manifest themselves in a stress situation where the integrity of the cellular DNA is threatened, it has been suggested that p53 is a "guardian of the genome". The presence of mutated p53 proteins in about 40 % of human tumours, all types taken together, reinforces this hypothesis and underlines the probably critical role which mutations of this gene play in the tumour development (for

reviews, see Montenarh, *Oncogene*, 7, 1673-1680, 1992; Oren, *FASEB J.*, 6, 3169-3176, 1992; Zambetti and Levine, *FASEB J.*, 7, 855-865, 1993).

The wild-type p53 protein is subject to a complex regulation which involves the control of its synthesis and of its breakdown as well as that of its intracellular location and of its post translational modifications (see the reviews cited above). The wild-type p53 protein is extremely unstable with a half-life of a few minutes. In contrast, some mutated proteins which accumulate at a high level in tumours have a significantly extended half-life. Little has been clearly established as regards the degradation of p53. Indeed, neither the intracellular sites of degradation, nor the number and the nature of the catabolic pathways taken, nor the peptide units labelling p53 for its degradation are known. To our knowledge, the only information available relates to the involvement of the enzyme E1 of the ubiquitin cycle under certain experimental conditions (Ciechanover et al., *Proc. Natl. Acad. Sci. USA* 88, 139-143, 1991; Chowdary et al., *Molec. Cell. Biol.* 14, 1997-2003, 1994). Moreover, it has been shown that certain proteolytic products derived from p53 may be presented in an antigenic manner.

The present invention results partly from the demonstration that the p53 proteins are substrates for calcium-dependent proteases: the calpains. It results



more particularly from the demonstration that the p53 proteins are degraded specifically by m-calpain or  $\mu$ -calpain. The present invention constitutes the first demonstration of a mechanism for regulating the cellular levels of the p53 proteins and thus offers a new particularly effective and specific approach for modulating the levels of this protein in pathological situations such as especially certain cancers.

In particular, the present invention describes a new approach for the treatment of cancer, based on the use of compounds which modulate the activity of calpains on the p53 proteins, which make it possible either to activate the degradation of the mutated p53 proteins, in order to block their tumorigenic effect and/or to enhance the presentation of immunogenic peptides, or to stabilize the wild-type p53 protein, in order to counterbalance the tumorigenic effect of the mutated proteins expressed in the tumours and/or in order to induce the apoptosis of the tumour cells.

A first subject of the invention therefore consists in the use of a compound capable of modulating the activity of calpain for the preparation of a pharmaceutical composition for the treatment of cancers.

Calpains are ubiquitous enzymes found in most mammalian cells (for a review, see Croall and deMartino, *Physiol. Rev.*, 71, 813-847, 1991). They are

essentially cytoplasmic but they can penetrate into the nucleus by virtue of the destruction of the nuclear envelope during mitosis or following certain stimuli.

As indicated above, the proteolytic activity of calpains is dependent on the presence of calcium.

The compounds capable of modulating the activity of calpain for the purposes of the present invention may be of several types.

They may be compounds capable of inhibiting the activity of the calpain on the p53 proteins. These compounds are particularly advantageous since they can be used to inhibit, at least in part, the degradation of the wild-type p53 protein. These compounds therefore make it possible to stabilize intracellularly the wild-type p53 protein and to counterbalance the effect of the mutated forms. Among the inhibitory compounds which can be used within the framework of the invention there may be mentioned the protease inhibitors (leupeptin, aprotinin, PMSF, and the like), the calcium chelators (EGTA, EDTA, and the like) or more specific inhibitors such as calpastatin or any fragment or derivative thereof. Calpastatin is a known inhibitor of the calpains. Its sequence has been described in the prior art (SEQ ID No. 1). A particularly advantageous embodiment of the present invention consists in transferring into the tumours a vector carrying all or part of the sequence encoding calpastatin. This approach is particularly adapted to the treatment of

cancers which always have a wild-type p53 allele, such as colic or bronchial carcinomas for example. Various fragments or derivatives of calpastatin can be used within the framework of the present invention. Such

5 fragments or derivatives may be any molecule obtained from the sequence SEQ ID No. 1 by modification(s) of a genetic and/or chemical nature, preserving the capacity to inhibit, at least in part, the activity of a calpain. Modification of a genetic and/or chemical

10 nature is understood to mean any mutation, deletion, substitution, addition and/or modification of one or more nucleotides. Such modifications may be carried out with various ends, especially that of preparing sequences adapted to expression in a specific type of

15 vector or host, that of reducing the size of the sequence so as to facilitate their cellular penetration, that of increasing the inhibitory activity, or, in a particularly advantageous manner, of increasing the selectivity of the inhibitor towards the

20 activity of the calpains on the degradation of the wild-type p53 protein.

Such modifications may be carried out, for example, by *in vitro* mutagenesis, by introduction of additional constituents or of synthetic sequences, or

25 by deletions or substitutions of the original constituents. When a derivative as defined above is prepared, its activity as inhibitor of the activity of the calpains on p53 proteins can be demonstrated in

several ways, and in particular by bringing into contact the said inhibitor and the various forms of p53 proteins, and then by detecting the degradation products obtained (see Examples 1 to 3). Any other  
5 technique known to persons skilled in the art can obviously be used to this effect.

In a specific embodiment of the present invention, all or part of calpastatin, or a nucleic acid encoding all or part of calpastatin is used as  
10 inhibitor. Still more particularly, a peptide comprising all or part of the sequence SEQ ID No. 1 or of a derivative thereof is used.

As regards more particularly the derivatives, there may be mentioned, by way of example, the compound  
15 of sequence SEQ ID No. 2, which corresponds to a fragment of calpastatin. There is advantageously used any derivative composed of the sequence SEQ ID No. 1 or 2 which is capable of specifically or preferentially inhibiting the degradation of the wild-type p53 protein  
20 by calpain.

The compounds capable of modulating the activity of calpain on the p53 proteins for the purposes of the present invention may also be derivative of calpain capable of specifically or  
25 preferentially degrading the mutated p53 proteins. Such derivatives are also very advantageous since they make it possible to activate the degradation of the mutated p53 proteins, in order to block their tumorigenic

effect and/or to increase the presentation of the immunogenic peptides, without significantly affecting the cellular levels of the wild-type p53 protein. Such derivatives may be obtained from calpain, by structural  
5 modification(s) of a genetic and/or chemical nature. The capacity of the derivatives thus obtained to specifically or preferentially degrade the mutated p53 proteins may then be demonstrated as described in Examples 1 to 3.

10 Preferably, the modulators used within the framework of the invention are proteins or polypeptides, or nucleic acid sequences encoding these polypeptides or proteins. Still more preferably, the modulatory compounds are proteins or polypeptides which  
15 are specific inhibitors of the activity of calpain on the wild-type p53 protein or forms of calpains, modified or otherwise, for specifically degrading the mutated p53 proteins.

In a particularly advantageous manner, the  
20 invention consists in the possibility of bringing about the expression in cancer cells having both a wild-type p53 allele and a mutated p53 allele of nucleic sequences encoding inhibitors of calpain, such as calpastatin or part of calpastatin, or forms of  
25 calpains, modified or otherwise, for specifically degrading the mutated p53 proteins.

The nucleic acid sequence used within the framework of the present invention may be administered

as such, in the form of naked DNA according to the technique described in Application WO 90/11092. It can also be administered in a form complexed, for example, with DEAE-dextran (Pagano et al., J. Virol. 1 (1967) 891), with nuclear proteins (Kaneda et al., Science 243 (1989) 375), with lipids (Felgner et al., PNAS 84 (1987) 7413), in the form of liposomes (Fraley et al., J. Biol. Chem. 255 (1980) 10431), and the like.

Preferably, the sequence used within the framework of the invention forms part of a vector. The use of such a vector indeed makes it possible to improve the administration of the nucleic acid into the cells to be treated, and also to increase its stability in the said cells, which makes it possible to obtain a lasting therapeutic effect. Furthermore, it is possible to introduce several nucleic acid sequences into the same vector, which also increases the efficacy of the treatment.

The vector used may be of various origin, as long as it is capable of transforming animal cells, preferably human cancer cells. In a preferred embodiment of the invention, a viral vector is used which may be chosen from adenoviruses, retroviruses, adeno-associated viruses (AAV) or the herpes virus.

In this regard, the subject of the present invention is any recombinant virus comprising, inserted into its genome, a nucleic acid encoding a compound capable of modulating the activity of calpain.

Preferably, the viruses used within the framework of the invention are defective, that is to say that they are incapable of replicating autonomously in the infected cell. Generally, the genome of the defective viruses used within the framework of the present invention therefore lacks at least the sequences necessary for the replication of the said virus in the infected cell. These regions may be either removed (completely or in part), or made nonfunctional, or substituted by other sequences and especially by the sequence encoding the modulator of the calpains. Preferably, the defective virus retains, nevertheless, the sequences of its genome which are necessary for the encapsidation of the viral particles.

As regards more particularly adenoviruses, various serotypes, whose structure and properties vary somewhat, have been characterized. Among these serotypes, the use of the type 2 or 5 human adenoviruses (Ad 2 or Ad 5) or of the adenoviruses of animal origin (see application FR 93 05954) is preferred within the framework of the present invention. Among the adenoviruses of animal origin which can be used within the framework of the present invention, there may be mentioned adenoviruses of canine, bovine, murine (example: MVA1, Beard et al., Virology 75 (1990) 81), ovine, porcine, avian or alternatively simian (example: SAV) origin. Preferably, the adenovirus of animal origin is a canine adenovirus,

or more preferably a CAV2 adenovirus [Manhattan strain or A26/61 (ATCC VR-800) for example]. Preferably, adenoviruses of human or canine or mixed origin are used within the framework of the invention.

5                    Preferably, the defective adenoviruses of the invention comprise the ITRs, a sequence allowing the encapsidation and the sequence encoding the modulator of the calpains. Still more preferably, in the genome of the adenoviruses of the invention, the E1 gene and  
10                    at least one of the genes E2, E4, L1-L5 are nonfunctional. The viral gene considered can be rendered non-functional by any technique known to persons skilled in the art, and especially by total suppression, by substitution or partial deletion, or by  
15                    addition of one or more bases in the gene(s) considered. Such modifications can be obtained in vitro (on the isolated DNA) or in situ, for example by means of genetic engineering techniques, or alternatively by treating with mutagenic agents.

20                    The defective recombinant adenoviruses according to the invention can be prepared by any technique known to persons skilled in the art (Levrero et al., Gene 101 (1991) 195, EP 185 573; Graham, EMBO J. 3 (1984) 2917). In particular, they can be  
25                    prepared by homologous recombination between an adenovirus and a plasmid carrying, inter alia, the DNA sequence encoding the modulator of the calpains. The homologous recombination occurs after co-transfection



of the said adenoviruses and plasmid into an appropriate cell line. The cell line used should preferably (i) be transformable by the said elements, and (ii) contain the sequences capable of complementing the defective adenovirus genome part, preferably in integrated form in order to avoid risks of recombination. As an example of a cell line, there may be mentioned the human embryonic kidney line 293 (Graham et al., J. Gen. Virol. 36 (1977) 59) which contains especially, integrated in its genome, the left hand part of the genome of an Ad5 adenovirus (12 %). Strategies for constructing vectors derived from adenoviruses have also been described in Applications Nos. FR 93 05954 and FR 93 08596.

Next, the adenoviruses which have multiplied are recovered and purified according to conventional molecular biology techniques as illustrated in the examples.

As regards the adeno-associated viruses (AAV), they are relatively small DNA viruses which become integrated into the genome of the cells which they infect, in a stable and site-specific manner. They are capable of infecting a broad spectrum of cells, without inducing any effect on cell growth, morphology or differentiation. Moreover, they do not seem to be involved in pathologies in man. The genome of the AAVs has been cloned, sequenced and characterized. It comprises about 4700 bases and contains, at each end,

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As regards the herpes viruses and the

retroviruses, the construction of recombinant vectors has been widely described in the literature: see especially Breakfield et al., New Biologist 3 (1991) 203; EP 453242, EP 178220, Bernstein et al. Genet. Eng. 7 (1985) 235; McCormick, BioTechnology 3 (1985) 689, and the like.

For carrying out the present invention, it is most particularly advantageous to use a defective recombinant retrovirus or adenovirus. These vectors indeed have particularly advantageous properties for the transfer of genes into tumour cells.

Advantageously, in the vectors of the invention, the sequence encoding the modulator of the calpains is placed under the control of signals allowing its expression in tumour cells. Preferably, these are heterologous expression signals, that is to say signals different from those which are naturally responsible for the expression of the modulator. They may be in particular sequences responsible for the expression of other proteins, or synthetic sequences. In particular, they may be promoter sequences of eukaryotic or viral genes. For example, they may be promoter sequences derived from the genome of the cell which it is desired to infect. Likewise, they may be promoter sequences derived from the genome of a virus, including the virus used. In this regard, the E1A, MLP, CMV, RSV-LTR promoters and the like may be mentioned for example. In addition, these expression sequences

may be modified by addition of activating or regulatory sequences or of sequences allowing a tissue-specific expression. It may indeed be particularly advantageous to use expression signals which are active specifically or predominantly in tumour cells, so that the DNA sequence is expressed or produces its effect only when the virus has effectively infected a tumour cell.

In a specific embodiment, the invention relates to a defective recombinant virus comprising a cDNA sequence encoding a modulator of the calpains under the control of a viral promoter, preferably chosen from the RSV-LTR and the CMV promoter.

Still in a preferred embodiment, the invention relates to a defective recombinant virus comprising a DNA sequence encoding a modulator of the calpains under the control of a promoter allowing predominant expression in tumour cells.

The expression is considered to be predominant for the purposes of the invention when, even if a residual expression is observed in other cell types, the expression levels are greater in the tumour cells.

The present invention also relates to any pharmaceutical composition comprising one or more defective recombinant viruses as described above. These pharmaceutical compositions may be formulated for administrations via the topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous,

intraocular or transdermal route and the like.

Preferably, the pharmaceutical compositions of the invention contain a vehicle pharmaceutically acceptable for an injectable formulation, especially for a direct  
5 injection into the patient's tumour. This may be in particular isotonic sterile solutions, or dry, especially freeze-dried, compositions which, upon addition, depending on the case, of sterile water or of physiological saline, allow the preparation of  
10 injectable solutions. Direct injection into the patient's tumour is advantageous because it makes it possible to concentrate the therapeutic effect at the level of the affected tissues.

The doses of defective recombinant virus  
15 which are used for the injection may be adapted according to various parameters, and especially according to the viral vector, the mode of administration used, the relevant pathology or alternatively the desired duration of the treatment. In  
20 general, the recombinant adenoviruses according to the invention are formulated and administered in the form of doses of between  $10^4$  to  $10^{14}$  pfu/ml, and preferably  $10^6$  to  $10^{10}$  pfu/ml. The term pfu ("plaque forming unit") corresponds to the infectivity of a virus solution, and  
25 is determined by infecting an appropriate cell culture and measuring, generally after 48 hours, the number of plaques of infected cells. The techniques for determining the pfu titre of a viral solution are well

documented in the literature. As regards the retroviruses, the compositions according to the invention may directly comprise the producing cells, for their implantation.

5           The present invention is particularly adapted to the treatment of cancers in which the mutated forms of p53 are observed. More specifically, the present invention is particularly advantageous for the treatment of cancers in which the wild-type and mutated  
10 alleles of p53 are present. Such cancers are especially colorectal cancer, breast cancer, lung cancer, gastric cancer, oesophageal cancer, B lymphomas, ovarian cancer, cancer of the bladder and the like.

          The present invention will be more fully  
15 described with the aid of the following Examples which should be considered as illustrative and nonlimiting.

#### Legend to the Figures

Figure 1: Study of the regulation of the p53 protein by calpain. The reaction is carried out in a final volume  
20 of 30  $\mu$ l, of which 1 comes from the translation mixture. Line 1: T0; line 2: 30 min in the presence of 1 mM Calcium + 20  $\mu$ g/ml Calpain; line 4: 30 min in the presence of 1 mM Calcium + 20  $\mu$ g/ml Calpain + 0.5 mg/ml calpastatin; line 5: 30 min in the presence of 1 mM  
25 Calcium + 20  $\mu$ g/ml Calpain + 10 mM EGTA; line 6: PBS; line 7: PBS + calcium; line 8: PBS + calpastatin.

#### General molecular biology techniques

The methods conventionally used in molecular

biology, such as preparative extractions of plasmid DNA, centrifugation of plasmid DNA in caesium chloride gradient, agarose or acrylamide gel electrophoresis, purification of DNA fragments by electroelution, phenol or phenol-chloroform extraction of proteins, ethanol or isopropanol precipitation of DNA in saline medium, transformation in *Escherichia coli* and the like, are well known to persons skilled in the art and are widely described in the literature [Maniatis T. et al., "Molecular Cloning, a Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982; Ausubel F.M. et al. (eds), "Current Protocols in Molecular Biology", John Wiley & Sons, New York, 1987].

The pBR322 and pUC type plasmids and the phages of the M13 series are of commercial origin (Bethesda Research Laboratories).

For the ligations, the DNA fragments can be separated according to their size by agarose or acrylamide gel electrophoresis, extracted with phenol or with a phenol/chloroform mixture, precipitated with ethanol and then incubated in the presence of phage T4 DNA ligase (Biolabs) according to the recommendations of the supplier.

The filling of the protruding 5' ends can be performed with the Klenow fragment of *E. coli* DNA polymerase I (Biolabs) according to the specifications of the supplier. The destruction of the protruding 3' ends is performed in the presence of phage T4 DNA

polymerase (Biolabs) used according to the recommendations of the manufacturer. The destruction of the protruding 5' ends is performed by a controlled treatment with S1 nuclease.

5                   Site-directed mutagenesis in vitro by synthetic oligodeoxynucleotides can be performed according to the method developed by Taylor et al. [Nucleic Acids Res. 13 (1985) 8749-8764] using the kit distributed by Amersham.

10                   The enzymatic amplification of the DNA fragments by the so-called PCR technique [Polymerase-catalyzed Chain Reaction, Saiki R.K. et al., Science 230 (1985) 1350-1354; Mullis K.B. and Faloona F.A., Meth. Enzym. 155 (1987) 335-350] can be performed using  
15 a DNA thermal cycler (Perkin Elmer Cetus) according to the specifications of the manufacturer.

                  The verification of the nucleotide sequences can be performed by the method developed by Sanger et al. [Proc. Natl. Acad. Sci. USA, 74 (1977) 5463-  
20 5467] using the kit distributed by Amersham.

### Examples

#### Example 1

                  This example shows that the addition of m-calpain to rabbit reticulocyte lysate induces the  
25 degradation of the wild-type p53 protein as well as that of certain mutated forms. This example also shows that inhibitors of calpains are capable of inhibiting the degradation of p53 and therefore of modulating the



activity of this protein.

1.1. Demonstration of the degradation: mouse and human wild-type p53 proteins as well as various mutated p53 proteins (human proteins C273, H273, H175, I247) were translated in the rabbit reticulocyte lysate. The proteins thus obtained are resistant to any degradation, even in the presence of a high concentration of calcium (cofactor essential for the calpains). The addition of bovine m-calpain (Sigma) to the reticulocyte lysate in the presence of calcium led to the rapid disappearance of the neosynthesized proteins and the appearance of proteolytic fragments which are resolvable by electrophoresis. The degradation resistance of other proteins such as dihydrofolate reductase or glyceraldehyde-3-phosphate dehydrogenase under the same experimental conditions indicates the substrate specificity of the reaction.

1.2. Use of inhibitors of calpain for modulating the levels of p53 proteins: in the above Example 1.1., it was shown that the addition of m-calpain induced degradation of the p53 proteins. In this example, in addition to m-calpain, various compounds were introduced into the medium in order to test their capacity to inhibit the activity of calpain. The results obtained show that the addition of a calcium chelator (EGTA) as well as of a peptide which is a specific inhibitor of the calpains (derivative of a physiological inhibitor, calpastatin; Maki et al., J.

Biol. Chem., 254, 18866-18869, 1989) are capable of inhibiting the degradation of the p53 proteins which is induced by the exogenous calpain.

#### Example 2

5                   In the preceding example, it was shown that the addition of exogenous calpain to a solution of p53 proteins brought about their degradation. This example shows that the degradation of the wild-type p53 protein as well as that of certain mutated forms may be induced  
10 by the endogenous calpains in cytoplasmic extracts. This example also shows that inhibitors of the calpains are capable, in the presence of endogenous calpain, of inhibiting the degradation of p53 and therefore of modulating the activity of this protein.

15                   2.1. Degradation by the endogenous calpains: mouse and human wild-type p53 proteins, as well as certain mutated forms (cf Example 1) were translated in the reticulocyte lysate and were then incubated in the presence of cytoplasmic extracts of Daudi or Jurkat  
20 human lymphoblastoid cells. The cytoplasmic extracts were prepared in the following manner: the cells (available at the ATCC) were cultured in DMEM medium supplemented with 10 % foetal calf serum. The cells were then harvested, washed in PBS buffer and then  
25 incubated for 5 min in a detergent-free hypotonic lysis buffer (HEPES 20 mM, pH 7.5; KOAc 10 mM; MgOAc 1.5 mM; 2 ml per  $5 \times 10^8$  cells). The lysis was completed using a Dounce homogenizer and then checked under a

microscope. The nuclei were then removed by centrifugation at 2000 g for 5 min, and the supernatants were centrifuged at 10,000 g for 1 hour (Beckman SW60). The cytoplasmic extracts were then aliquoted in an amount of 5 to 12 mg/ml.

When the lysate of reticulocytes was incubated in the presence of cytoplasmic extracts, in the absence of calcium, no degradation was observed. On the other hand, in the presence of calcium, a very rapid degradation of the p53 proteins was observed, with the appearance of a characteristic proteolytic product profile similar to that obtained in Example 1. This experiment indeed shows that the p53 proteins are degraded by the endogenous calpains.

2.2. Use of calpain inhibitors to modulate the levels of p53 proteins: the chelation of calcium by EGTA, as well as the use of a whole range of protease inhibitors (leupeptin, aprotinin, soybean trypsin inhibitor and PMSF) and especially the peptide calpastatin show that the degradation of these proteins is dependent on the calpains of the cytoplasmic extract, and that various compounds capable of modulating the activity of the calpains may be used to regulate the p53 protein levels.

### Example 3

This example demonstrates that the mouse and human wild-type p53 proteins are direct substrates for the calpains in the cytoplasmic extracts.

Examples 1 and 2 show that the calpains can induce the degradation of p53 in complex reaction mixtures. These experiments do not exclude, however, that under the conditions used, the calpains activate secondary proteases which are those which actually act on p53. In this example, the following experiment was conducted: (1) the mouse and human wild-type p53 proteins neosynthesized in the rabbit reticulocyte lysate were incubated for 30 minutes in the presence of a cytoplasmic extract of Daudi cells as well as in the presence of calcium to activate the calpains as in Example 2, (2) p53 protein was then added to the reaction mixture and the reaction was continued for 30 minutes under conditions permissive (same reaction conditions) or otherwise (addition either of EGTA to chelate the calcium, or of calpastatin peptide) for the calpains. In the presence of calcium, the newly added p53 protein is completely degraded, indicating that the protease activity is functional throughout the experiment. When the calpains are inhibited by the presence of EGTA or, more significantly, of the calpastatin peptide, the newly added p53 protein is, on the other hand, no longer degraded. This latter observation therefore excludes the possibility that in the first part of the experiment, the calpains induced a second protease responsible for the degradation of p53 (Figure 1).

Example 4

This example describes the construction of a recombinant adenovirus comprising a nucleic acid sequence encoding calpastatin. This adenovirus is constructed by homologous recombination between the defective adenovirus Ad-dl1324 and a plasmid carrying the sequence SEQ ID No. 1 under the control of the RSV promoter.

## 4.1. Construction of the plasmid SEQ ID No. 1

The plasmid SEQ ID No. 1 comprises the sequence encoding calpastatin under the control of the RSV-LTR promoter, as well as regions of the adenovirus which allow homologous recombination. It is constructed by inserting the sequence SEQ ID No. 1 into the plasmid pAd.RSV $\beta$ gal. The plasmid pAd.RSV $\beta$ Gal contains, in the 5'→3' orientation,

- the PvuII fragment corresponding to the left hand end of the Ad5 adenovirus comprising: the ITR sequence, the replication origin, the encapsidation signals and the enhancer E1A;

- the gene encoding  $\beta$ -galactosidase under the control of the RSV promoter (Rous sarcoma virus),

- a second fragment of the Ad5 adenovirus genome which allows homologous recombination between the plasmid pAd.RSV $\beta$ Gal and the adenovirus dl1324. The plasmid pAd.RSV $\beta$ Gal has been described by Stratford-Perricaudet et al. (J. Clin. Invest. 90 (1992) 626).

#### 4.2. Construction of the recombinant adenovirus

The vector described in 4.1. is linearized and cotransfected with a deficient adenoviral vector into the helper cells (line 293) providing in trans the functions encoded by the adenovirus E1 regions (E1A and E1B).

More specifically, the recombinant adenovirus is obtained by homologous recombination in vivo between the mutant adenovirus Ad-dl1324 (Thimmappaya et al., Cell 31 (1982) 543) and the vector described in Example 4.1., according to the following procedure: the plasmid SEQ ID No. 1 and the adenovirus Ad-dl1324, linearized by the enzyme ClaI, are cotransfected into the line 293 in the presence of calcium phosphate, so as to allow the homologous recombination. The recombinant adenoviruses thus generated are then selected by plaque purification. After isolation, the recombinant adenovirus DNA is amplified in the cell line 293, leading to a culture supernatant containing the unpurified recombinant defective adenovirus having a titre of about  $10^{10}$  pfu/ml.

The viral particles are purified by centrifugation on a caesium chloride gradient according to known techniques (see especially Graham et al., Virology 52 (1973) 456). The adenovirus obtained may be stored at  $-80^{\circ}\text{C}$  in 20 % glycerol.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

- (A) NAME: RHONE-POULENC RORER S.A.  
(B) STREET: 20, avenue Raymond ARON  
(C) CITY: ANTONY  
(E) COUNTRY: FRANCE  
(F) POSTAL CODE: 92165

(ii) TITLE OF THE INVENTION: Method of treating  
cancer by regulation of the p53 protein.

(iii) NUMBER OF SEQUENCES: 2

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Tape  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version  
#1.30 (EPO)

## (2) INFORMATION FOR SEQ ID No.: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2085 base pairs  
(B) TYPE: nucleotide  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..2085

5

(D) OTHER INFORMATION: /product = "human  
calpastatin"

(xi) SEQUENCE DESCRIPTION: SEQ ID No.: 1:

ATG GAA GGA CCA CAT CTT CCT AAC AAG AAA AAA CAC AAA AAA CAG GCT	48
Met Glu Gly Pro His Leu Pro Asn Lys Lys Lys His Lys Lys Gln Ala	
1 5 10 15	
GTA AAA ACA GAA CCT GAG AAG AAG TCA CAG TCA ACC AAG CTG TCT GTG	96
Val Lys Thr Glu Pro Glu Lys Lys Ser Gln Ser Thr Lys Leu Ser Val	
20 25 30	
GTT CAT GAG AAA AAA TCC CAA GAA GGA AAG CCA AAA GAA CAC ACA GAG	144
Val His Glu Lys Lys Ser Gln Glu Gly Lys Pro Lys Glu His Thr Glu	
35 40 45	
CCA AAA AGC CTA CCC AAG CAG GCA TCA GAT ACA GGA AGT AAC GAT GCT	192
Pro Lys Ser Leu Pro Lys Gln Ala Ser Asp Thr Gly Ser Asn Asp Ala	
50 55 60	
CAC AAT AAA AAA GCA GTT TCC AGA TCA GCT GAA CAG CAG CCA TCA GAG	240
His Asn Lys Lys Ala Val Ser Arg Ser Ala Glu Gln Gln Pro Ser Glu	
65 70 75 80	
AAA TCA ACA GAA CCA AAG ACT AAA CCA CAA GAC ATG ATT TCT GCT GGT	288
Lys Ser Thr Glu Pro Lys Thr Lys Pro Gln Asp Met Ile Ser Ala Gly	
85 90 95	
GGA GAG AGT GTT GCT GGT ATC ACT GCA ATA TCT GGC AAG CCG GGT GAC	336
Gly Glu Ser Val Ala Gly Ile Thr Ala Ile Ser Gly Lys Pro Gly Asp	
100 105 110	
AAG AAA AAA GAA AAG AAA TCA TTA ACC CCA GCT GTG CCA GTT GAA TCT	384
Lys Lys Lys Glu Lys Lys Ser Leu Thr Pro Ala Val Pro Val Glu Ser	
115 120 125	
AAA CCG GAT AAA CCA TCG GGA AAG TCA GGC ATG GAT GCT GCT TTG GAT	432
Lys Pro Asp Lys Pro Ser Gly Lys Ser Gly Met Asp Ala Ala Leu Asp	
130 135 140	
GAC TTA ATA GAT ACT TTA GGA GGA CCT GAA GAA ACT GAA GAA GAA AAT	480
Asp Leu Ile Asp Thr Leu Gly Gly Pro Glu Glu Thr Glu Glu Glu Asn	
145 150 155 160	
ACA ACG TAT ACT GGA CCA GAA GTT TCA GAT CCA ATG AGT TCC ACC TAC	528
Thr Thr Tyr Thr Gly Pro Glu Val Ser Asp Pro Met Ser Ser Thr Tyr	
165 170 175	
ATA GAG GAA TTG GGT AAA AGA GAA GTC ACA ATT CCT CCA AAA TAT AGG	576
Ile Glu Glu Leu Gly Lys Arg Glu Val Thr Ile Pro Pro Lys Tyr Arg	
180 185 190	
GAA CTA TTG GCT AAA AAG GAA GGG ATC ACA GGG CCT CCT GCA GAC TCT	624
Glu Leu Leu Ala Lys Lys Glu Gly Ile Thr Gly Pro Pro Ala Asp Ser	
195 200 205	



TCA	AAA	CCC	ATA	GGG	CCA	GAT	GAT	GCT	ATA	GAC	GCC	TTG	TCA	TCT	GAC	672
Ser	Lys	Pro	Ile	Gly	Pro	Asp	Asp	Ala	Ile	Asp	Ala	Leu	Ser	Ser	Asp	
210						215					220					
TTC	ACC	TGT	GGG	TCG	CCT	ACA	GCT	GCT	GGA	AAG	AAA	ACT	GAA	AAA	GAG	720
Phe	Thr	Cys	Gly	Ser	Pro	Thr	Ala	Ala	Gly	Lys	Lys	Thr	Glu	Lys	Glu	
225					230				235						240	
GAA	TCT	ACA	GAA	GTT	TTA	AAA	GCT	CAG	TCA	GCA	GGG	ACA	GTC	AGA	AGT	768
Glu	Ser	Thr	Glu	Val	Leu	Lys	Ala	Gln	Ser	Ala	Gly	Thr	Val	Arg	Ser	
				245					250					255		
GCT	GCT	CCA	CCC	CAA	GAG	AAG	AAA	AGA	AAG	GTG	GAG	AAG	GAT	ACA	ATG	816
Ala	Ala	Pro	Pro	Gln	Glu	Lys	Lys	Arg	Lys	Val	Glu	Lys	Asp	Thr	Met	
			260					265					270			
AGT	GAT	CAA	GCA	CTC	GAG	GCT	CTG	TCG	GCT	TCA	CTG	GGC	ACC	CGG	CAA	864
Ser	Asp	Gln	Ala	Leu	Glu	Ala	Leu	Ser	Ala	Ser	Leu	Gly	Thr	Arg	Gln	
		275					280					285				
GCA	GAA	CCT	GAG	CTC	GAC	CTC	CGC	TCA	ATT	AAG	GAA	GTC	GAT	GAG	GCA	912
Ala	Glu	Pro	Glu	Leu	Asp	Leu	Arg	Ser	Ile	Lys	Glu	Val	Asp	Glu	Ala	
	290					295					300					
AAA	GCT	AAA	GAA	GAA	AAA	CTA	GAG	AAG	TGT	GGT	GAG	GAT	GAT	GAA	ACA	960
Lys	Ala	Lys	Glu	Glu	Lys	Leu	Glu	Lys	Cys	Gly	Glu	Asp	Asp	Glu	Thr	
305					310				315					320		
ATC	CCA	TCT	GAG	TAC	AGA	TTA	AAA	CCA	GCC	ACG	GAT	AAA	GAT	GGA	AAA	1008
Ile	Pro	Ser	Glu	Tyr	Arg	Leu	Lys	Pro	Ala	Thr	Asp	Lys	Asp	Gly	Lys	
				325					330					335		
CCA	CTA	TTG	CCA	GAG	CCT	GAA	GAA	AAA	CCC	AAG	CCT	CGG	AGT	GAA	TCA	1056
Pro	Leu	Leu	Pro	Glu	Pro	Glu	Glu	Lys	Pro	Lys	Pro	Arg	Ser	Glu	Ser	
			340					345					350			
GAA	CTC	ATT	GAT	GAA	CTT	TCA	GAA	GAT	TTT	GAC	CGG	TCT	GAA	TGT	AAA	1104
Glu	Leu	Ile	Asp	Glu	Leu	Ser	Glu	Asp	Phe	Asp	Arg	Ser	Glu	Cys	Lys	
		355					360					365				
GAG	AAA	CCA	TCT	AAG	CCA	ACT	GAA	AAG	ACA	GAA	GAA	TCT	AAG	GCC	GCT	1152
Glu	Lys	Pro	Ser	Lys	Pro	Thr	Glu	Lys	Thr	Glu	Glu	Ser	Lys	Ala	Ala	
	370					375					380					
GCT	CCA	GCT	CCT	GTG	TCG	GAG	GCT	GTG	TCT	CGG	ACC	TCC	ATG	TGT	AGT	1200
Ala	Pro	Ala	Pro	Val	Ser	Glu	Ala	Val	Ser	Arg	Thr	Ser	Met	Cys	Ser	
385					390					395					400	
ATA	CAG	TCA	GCA	CCC	CCT	GAG	CCG	GCT	ACC	TTG	AAG	GGC	ACA	GTG	CCA	1248
Ile	Gln	Ser	Ala	Pro	Pro	Glu	Pro	Ala	Thr	Leu	Lys	Gly	Thr	Val	Pro	
				405				410						415		
GAT	GAT	GCT	GTA	GAA	GCC	TTG	GCT	GAT	AGC	CTG	GGG	AAA	AAG	GAA	GCA	1296
Asp	Asp	Ala	Val	Glu	Ala	Leu	Ala	Asp	Ser	Leu	Gly	Lys	Lys	Glu	Ala	
			420				425					430				
GAT	CCA	GAA	GAT	GGA	AAA	CCT	GTG	ATG	GAT	AAA	GTC	AAG	GAG	AAG	GCC	1344
Asp	Pro	Glu	Asp	Gly	Lys	Pro	Val	Met	Asp	Lys	Val	Lys	Glu	Lys	Ala	
		435					440				445					
AAA	GAA	GAA	GAC	CGT	GAA	AAG	CTT	GGT	GAA	AAA	GAA	GAA	ACA	ATT	CCT	1392
Lys	G	Glu	Asp	Arg	Glu	Lys	Leu	Gly	Glu	Lys	Glu	Glu	Thr	Ile	Pro	
	450					455					460					
CCT	GAT	TAT	AGA	TTA	GAA	GAG	GTC	AAG	GAT	AAA	GAT	GGA	AAG	CCA	CTC	1440
Pro	Asp	Tyr	Arg	Leu	Glu	Glu	Val	Lys	Asp	Lys	Asp	Gly	Lys	Pro	Leu	
465					470				475						480	
CTG	CCA	AAA	GAG	TCT	AAG	GAA	CAG	CTT	CCA	CCC	ATG	AGT	GAA	GAC	TTC	1488
Leu	Pro	Lys	Glu	Ser	Lys	Glu	Gln	Leu	Pro	Pro	Met	Ser	Glu	Asp	Phe	
				485					490					495		

CTT	CTG	GAT	GCT	TTG	TCT	GAG	GAC	TTC	TCT	GGT	CCA	CAA	AAT	GCT	TCA	1536
Leu	Leu	Asp	Ala	Leu	Ser	Glu	Asp	Phe	Ser	Gly	Pro	Gln	Asn	Ala	Ser	
			500					505					510			
TCT	CTT	AAA	TTT	GAA	GAT	GCT	AAA	CTT	GCT	GCT	GCC	ATC	TCT	GAA	GTG	1584
Ser	Leu	Lys	Phe	Glu	Asp	Ala	Lys	Leu	Ala	Ala	Ala	Ile	Ser	Glu	Val	
		515					520					525				
GTT	TCC	CAA	ACC	CCA	GCT	TCA	ACG	ACC	CAA	GCT	GGA	GCC	CCA	CCC	CGT	1632
Val	Ser	Gln	Thr	Pro	Ala	Ser	Thr	Thr	Gln	Ala	Gly	Ala	Pro	Pro	Arg	
	530					535					540					
GAT	ACC	TCG	CAG	AGT	GAC	AAA	GAC	CTC	GAT	GAT	GCC	TTG	GAT	AAA	CTC	1680
Asp	Thr	Ser	Gln	Ser	Asp	Lys	Asp	Leu	Asp	Asp	Ala	Leu	Asp	Lys	Leu	
545					550				555						560	
TCT	GAC	AGT	CTA	GGA	CAA	AGG	CAG	CCT	GAC	CCA	GAT	GAG	AAC	AAA	CCA	1728
Ser	Asp	Ser	Leu	Gly	Gln	Arg	Gln	Pro	Asp	Pro	Asp	Glu	Asn	Lys	Pro	
			565						570					575		
ATG	GGA	GAT	AAA	GTA	AAG	GAA	AAA	GCT	AAA	GCT	GAA	CAT	AGA	GAC	AAG	1776
Met	Gly	Asp	Lys	Val	Lys	Glu	Lys	Ala	Lys	Ala	Glu	His	Arg	Asp	Lys	
			580					585					590			
CTT	GGA	GAA	AGA	GAT	GAC	ACT	ATC	CCA	CCT	GAA	TAC	AGA	CAT	CTC	CTG	1824
Leu	Gly	Glu	Arg	Asp	Asp	Thr	Ile	Pro	Pro	Glu	Tyr	Arg	His	Leu	Leu	
		595					600					605				
GAT	GAT	AAT	GGA	CAG	GAC	AAA	CCA	GTG	AAG	CCA	CCT	ACA	AAG	AAA	TCA	1872
Asp	Asp	Asn	Gly	Gln	Asp	Lys	Pro	Val	Lys	Pro	Pro	Thr	Lys	Lys	Ser	
	610					615					620					
GAG	GAT	TCA	AAG	AAA	CCT	GCA	GAT	GAC	CAA	GAC	CCC	ATT	GAT	GCT	CTC	1920
Glu	Asp	Ser	Lys	Lys	Pro	Ala	Asp	Asp	Gln	Asp	Pro	Ile	Asp	Ala	Leu	
625					630				635						640	
TCA	GGA	GAT	CTG	GAC	AGC	TGT	CCC	TCC	ACT	ACA	GAA	ACC	TCA	CAG	AAC	1968
Ser	Gly	Asp	Leu	Asp	Ser	Cys	Pro	Ser	Thr	Thr	Glu	Thr	Ser	Gln	Asn	
			645					650						655		
ACA	GCA	AAG	GAT	AAG	TGC	AAG	AAG	GCT	GCT	TCC	AGC	TCC	AAA	GCA	CCT	2016
Thr	Ala	Lys	Asp	Lys	Cys	Lys	Lys	Ala	Ala	Ser	Ser	Ser	Lys	Ala	Pro	
		660						665					670			
AAG	AAT	GGA	GGT	AAA	GCG	AAG	GAT	TCA	GCA	AAG	ACA	ACA	GAG	GAA	ACT	2064
Lys	Asn	Gly	Gly	Lys	Ala	Lys	Asp	Ser	Ala	Lys	Thr	Thr	Glu	Glu	Thr	
		675					680					685				
TCC	AAG	CCA	AAA	GAT	GAC	TAA										2085
Ser	Lys	Pro	Lys	Asp	Asp	*										
	690					695										

## (2) INFORMATION FOR SEQ ID NO.: 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:

(A) ORGANISM: homo sapiens

(A) NAME/KEY: CDS

(B) LOCATION: 1..399

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TCA Ser	GGC Gly	ATG Met	GAT Asp	GCT Ala	GCT Ala	TTG Leu	GAT Asp	GAC Asp	TTA Leu	ATA Ile	GAT Asp	ACT Thr	TTA Leu	GGA Gly	GGA Gly	48		
				700				705				710						
CCT Pro	GAA Glu	GAA Glu	ACT Thr	GAA Glu	GAA Glu	GAA Glu	AAT Asn	ACA Thr	ACG Thr	TAT Tyr	ACT Thr	GGA Gly	CCA Pro	GAA Glu	GTT Val	96		
				715				720				725						
TCA Ser	GAT Asp	CCA Pro	ATG Met	AGT Ser	TCC Ser	ACC Thr	TAC Tyr	ATA Ile	GAG Glu	GAA Glu	TTG Leu	GGT Gly	AAA Lys	AGA Arg	GAA Glu	144		
				730				735				740						
GTC Val	ACA Thr	ATT Ile	CCT Pro	CCA Pro	AAA Lys	TAT Tyr	AGG Arg	GAA Glu	CTA Leu	TTG Leu	GCT Ala	AAA Lys	AAG Lys	GAA Glu	GGG Gly	192		
				745				750				755						
ATC Ile	ACA Thr	GGG Gly	CCT Pro	CCT Pro	GCA Ala	GAC Asp	TCT Ser	TCA Ser	AAA Lys	CCC Pro	ATA Ile	GGG Gly	CCA Pro	GAT Asp	GAT Asp	240		
				760				765				770				775		
GCT Ala	ATA Ile	GAC Asp	GCC Ala	TTG Leu	TCA Ser	TCT Ser	GAC Asp	TTC Phe	ACC Thr	TGT Cys	GGG Gly	TCG Ser	CCT Pro	ACA Thr	GCT Ala	288		
				780				785				790						
GCT Ala	GGA Gly	AAG Lys	AAA Lys	ACT Thr	GAA Glu	AAA Lys	GAG Glu	GAA Glu	TCT Ser	ACA Thr	GAA Glu	GTT Val	TTA Leu	AAA Lys	GCT Ala	336		
				795				800				805						
CAG Gln	TCA Ser	GCA Ala	GGG Gly	ACA Thr	GTC Val	AGA Arg	AGT Ser	GCT Ala	GCT Ala	CCA Pro	CCC Pro	CAA Gln	GAG Glu	AAG Lys	AAA Lys	384		
				810				815				820						
AGA Arg	AAG Lys	GTG Val	GAG Glu	AAG Lys												399		
				825														

## CLAIMS

1. Use of a compound capable of modulating the activity of calpain for the preparation of a pharmaceutical composition for the treatment of cancer.

5           2. Use according to claim 1, characterized in that the compound is a protein or a polypeptide which is an inhibitor of the activity of calpain, or a nucleic acid sequence encoding such a polypeptide or protein.

10           3. Use according to claim 2, characterized in that the compound is a protein or a polypeptide which is a specific inhibitor of the activity of calpain on the wild-type p53 protein, or a nucleic acid sequence encoding such a polypeptide or protein.

15           4. Use according to claim 2 or 3, characterized in that the nucleic acid is part of a vector.

          5. Use according to claim 4, characterized in that the nucleic acid is part of a viral vector,  
20 chosen from adenoviruses, retroviruses and adeno-associated viruses.

          6. Use according to claim 4, characterized in that the nucleic acid is part of a lipid liposomal vector.

25           7. Use according to one of the preceding claims, characterized in that the compound is a nucleic acid encoding all or part of calpastatin.

8. Use according to claim 7, characterized in that the nucleic acid comprises all or part of the sequence SEQ ID No. 1 or a derivative thereof.

9. Use according to claim 8, characterized in that the nucleic acid is chosen from the sequences SEQ ID No. 1 and 2.

10. Use according to claim 8, characterized in that the nucleic acid is chosen from the derivatives of the sequences SEQ ID No. 1 or 2 encoding specific inhibitors of the degradation of the wild-type p53 protein.

11. Use according to one of claims 1 to 6, characterized in that the compound is a derivative of calpain capable of specifically degrading the mutated p53 proteins.

12. Viral vector comprising a nucleic acid sequence encoding a protein or a polypeptide which is an inhibitor of the activity of calpain.

13. Vector according to claim 12, characterized in that it is chosen from the adenoviruses, retroviruses and adeno-associated viruses.

14. Vector according to either of claims 12 or 13, characterized in that it comprises a sequence encoding all or part of calpastatin.

15. Vector according to claim 12, characterized in that it comprises a sequence encoding a derivative of calpain capable of specifically

degrading the mutated p53 proteins.

16. Pharmaceutical composition comprising a nucleic acid sequence encoding all or part of calpastatin or a derivative of calpain capable of specifically degrading the mutated p53 proteins.
- 5

17. Composition according to claim 16, formulated for intra-tumour administration.

10

## 15

20

## 25

The invention concerns, in particular, the use of a compound capable of modulating calpaine activity.

HUMAN

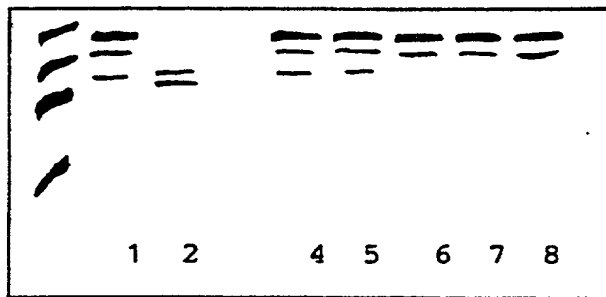
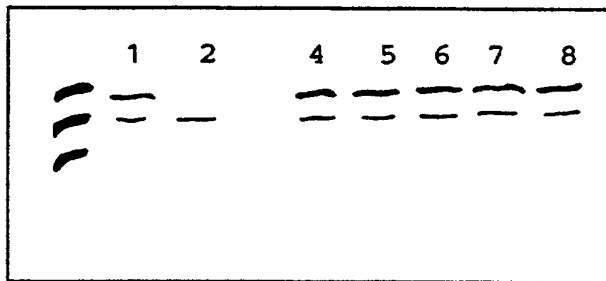


Fig 1 a



MOUSE

Figure 1 b



DECLARATION AND POWER OF ATTORNEY  
FOR PATENT APPLICATION

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought, by entry into the U.S. national stage of examination, on the invention entitled

**METHOD OF CANCER TREATMENT BY P53 PROTEIN CONTROL**

the international specification of which was filed on May 22, 1995 as Application Serial No. PCT/FR95/00670 which notice of transmission was given on December 7, 1995, by the International Bureau. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of a foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Applications(s)			Priority Claimed	
<u>FR94/06583</u>	<u>France</u>	<u>31 May 1994</u>	<u>X</u>	<u>      </u>
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
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(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT International filing date of this application:

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(Application Serial No.)	(Filing Date)	(Status-Patented, Pending or Abandoned)
<hr/>		
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(Application Serial No.)	(Filing Date)	(Status-Patented, Pending or Abandoned)

00453002650460

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I (We) hereby appoint as my (our) attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

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Raymond S. Parker, III	Reg. No. 34,893
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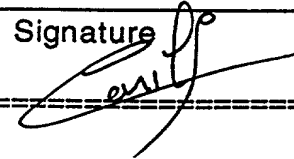
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State (Zip) or Country	State (Zip) or Country

Date  
November 8<sup>th</sup>, 1996

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Date November 4th, 1996

Signature  
[Signature]

661200 "02050460

In re the Application of:	Carillo <i>et al.</i>	Group Art Unit:
Serial No.	To Be Assigned	Examiner:
Filed:	Concurrently Herewith	

To: The Honorable Commissioner for Patents and Trademarks  
Washington, D.C. 20231

Dear Sir:

Please send all correspondence to:

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500 Arcola Road; P.O. Box 5093  
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Respectfully submitted,

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Dated: 9/22/99

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